**Supplemental Information for**

**Control of Drosophila wing size by morphogen range and hormonal gating.**

Joseph Parker and Gary Struhl

Corresponding authors: Joseph Parker, joep@caltech.edu, and Gary Struhl, gs20@columbia.edu

**Supplemental Information for**

**This PDF file includes:**

Supplementary Information Text

Figures S1-S5

Tables S1 and S2

SI References

## Supplemental Information Text

### Transgenes and chromosomes (Flybase designations in Table S1)

Gal4 and Gal80 transgenes: *phm.Gal4*, *C765.Gal4, Act>CD2>Gal4*, *ap.Gal4*, $Gal80^{ts}$

Lex::V16 and LexOP transgenes: $LexOP.fh^{RNAi}$ and *phm.LexA:VP16* (this study)

UAS transgenes: *UAS>CD2,y+>dpp*, *UAS.dpp-GFP* (gift of Myriam Zecca), *UAS>CD2,y+>Nrt-wg*, $UAS.fh^{RNAi}$, $UAS.EcR^{RNAi}$, $UAS.Usp^{RNAi}$, $UAS.EcR\text{-}B1^{DN}$, *UAS-GFP*.

Reporter transgenes: *dpp.lacZ*, *5XVgQE.DsRed* (1)

Mutant chromosomes: *FRT42D* $ptc^{16}$, *FRT42D* $Df(2R)en^{E}$, *FRT82B* $wts^{P2}$, $wg^{spd\text{-}flg}$

Cell marker chromosomes *arm.lacZ FRT40A*, *FRT42D Ubi.GFP*, *FRT42D Tubα1.DsRed* (1), *FRT 82B Ubi.GFP*.

New transgenes generated in this study (*phm.lexA:VP16*, $LexOP.fh^{RNAi}$ ).

*phm.lexA:VP16***.** A 1.2 kb fragment of the *phantom* enhancer (2) was inserted immediately upstream of the minimal *hsp43* promoter of the AttB vector pHS43-AttB (3) followed downstream by the LexA:VP16 coding sequence (4) and the resulting *phm.lexA:VP16* transgene was introduced into the genome via phiC31 integrase-mediated recombination at the attP40 [25C; (5)] and attJ5 [86F (6)] docking sites.

$LexOP.fh^{RNAi}$**.** A DNA fragment containing a multimerised LexA operator sequences followed by a TATA box, polylinker and SV40 poly-A tail was excised from pLOT (4) and inserted in place of the corresponding portion of pUAS-AttB (6) to make pLexOP-AttB. A DNA fragment containing the first 391 nucleotides of a full length *fh* cDNA (AT09528; Berkeley *Drosophila* Genome Project) was then inserted as a tandem, inverted repeat into the polylinker of pLexOP-AttB and resulting $LexOP.fh^{RNAi}$ transgene introduced at the attP40 [25C; (5)] and attJ5 [86F (6)] docking sites.

### Generation and analysis of stalled discs.

To generate larvae with imaginal discs destined to stall, we used transgenes that express the coding sequence for either LexA:VP16 or Gal4 under the control of a *phantom* (*phm*) gene

enhancer (*phm.LexA:VP16* or *phm.Gal4*) to drive expression of *LexOP* or *UAS* transgenes encoding RNAi against transcripts of the *frataxin* (*fh*) gene (*LexOP.fh*$^{RNAi}$ or *UAS.fh*$^{RNAi}$) in the larval prothoracic gland (7, 8). In all experiments, larvae carrying the stated combinations of transgenes, cell markers, FRTs and mutant alleles were generated by standard genetic crosses between parents in which the desired genetic elements (Table S1) were either homozygous or maintained over balancers carrying the dominant mutation Tubby (TM6B, or SM5-TM6B), allowing the selection of progeny of the desired genotypes (Table S2) as non-Tubby larvae.

Larvae were grown at low density on yeast-supplemented food (at 25$^{o}$C, unless otherwise stated). For the time courses of growth arrest (Figs. 2C-E) and 20E titer (Fig. 1F), fertilized eggs were collected for 4 hr. intervals following an initial 2 hr. pre-collection. Under these conditions discs destined to stall reach full size and arrest ~6-7 days after egg laying (AEL). To supply ectopic morphogen to stalled discs, *phm.LexA:VP16 LexOP.fh*$^{RNAi}$ larvae carrying a given *UAS>CD2, y+>morphogen* transgene as well as *hsp70.flp*, the imaginal disc specific driver *C765.Gal4*, and as needed, the *5XQE.DsRed* reporter were heat-shocked for 1 hr. at 37°C at 6-7 days AEL to excise the *>CD2, y+>* cassette and pepper the disc with cells that constitutively express the morphogen [for Wg, a short range form, Nrt-Wg (9–11), was used as corresponding experiments using a *UAS>CD2,y+>wg* transgene encoding native Wg failed owing to animal toxicity]. Discs were dissected 1–3 days later to observe proliferation using EdU incorporation, or 5–7 days later to assess the change in wing size (as monitored by the domain of *5XQE.DsRed* or native Vg expression). To monitor the patterns of cell proliferation via twin-spot lineage tracing, standard combinations of FRTs and accompanying cell marker transgenes were also included (specifically, *FRT40 arm.lacZ*, *FRT42 arm.lacZ*, *FRT42.Tub.DsRed* or *FRT82 Ubi.GFP*), each *in trans* to the same FRT without the cell marker, and larvae were heat shocked twice for 1 hr. at 37 °C, with an intervening recovery period of 3 hrs. To create marked *ptc*$^{-}$ or *en*$^{-}$ clones in stalled discs, larvae transheterozygous for the appropriate *FRT42 mutant* and *FRT42 cell marker* combinations were heat shocked either 5 (*ptc*$^{-}$) or 4 (*en*$^{-}$) days AEL, after which most cells divide only once or a few times before growth arrests (Fig. 2E); the final cell divisions in such discs are required to generate mutant daughter cells following heat-shock induced mitotic recombination in mother cells. The discs from such larvae were fixed and stained 5–7 days later to assess wing size and the growth of lineage trace clones.

**Antibody staining.**

Larvae were dissected in PBS, fixed in 4% PFA and stained using mouse anti-Wg (1:30; DSHB), mouse anti-Ptc (1:150; DHSB), mouse anti-En (1:10; DSHB), mouse anti-Dl (1:20; DSHB), mouse anti-Br core (1:100; DSHB), rabbit anti-ß-galactosidase (1:6,000; Cappel), rabbit anti-Hh (1:500; T. Tabata), rabbit and guinea pig anti-Dll (both 1:3,000; R. Mann), guinea pig anti-Vg (1:500) and guinea pig anti-pMAD (1:1000; E. Laufer) antisera, followed by Alexa Fluor secondary antibodies (Invitrogen; 1/3000) and the DNA counterstain Hoechst.

**Edu labelling of S-phase nuclei**

Larvae were dissected and incubated for 45 min. in 1 μM EdU in Schneider's medium, fixed for 20 min. in 4% PFA and stained with primary and secondary antibodies. After staining, a Click-iT reaction was performed in 250 μl according to the instructions of the manufacturer (Thermofisher).

Note that EdU signal intensity depends on the proportion of nuclei in S-phase during the 45 min. EdU incubation period, as well as the rate and duration of incorporation during that period. Accordingly, we interpret the progressive decline in EdU signal intensity after discs begin to stall as a reflection of both the reduced number of cells in S phase as well as the progressive slow-down in DNA synthesis in these cells, until the level of incorporation falls beneath the threshold necessary to generate detectable EdU signal. After this time, any residual proliferative growth that occurs is apparent only in a modest increase in the size of the disc before complete growth arrest (as in Figs. 2E).

**Quantitation.**

To assess disc size (Fig. 2C), confocal images of Hoechst-stained discs derived from female larvae were quantified by pixel count in Adobe Photoshop using the magic wand tool. All discs were measured "blind", without knowledge of the genotype or disc age.

For comparisons of gene expression between normal and stalled discs, or between stalled discs from larvae fed or not fed 20E, control discs were distinguished from experimentals by *ap.Gal4* driven *UAS.GFP* expression and grown in parallel with experimental discs, fixed and stained in

the same tube, mounted under the same coverslip and imaged using the same Leica SP5 confocal settings (as in Fig. 1G,H).

For all experiments, at least 15 and typically >30 discs of each genotype and condition were assayed. The results, as presented in the Figures, are representative of the great majority (>90%) of discs examined.

**20E feeding assay.**

**Feeding assay:** 20E-containing food (12) was made by preparing yeast paste supplemented with 20E (Sigmal). 4.5 g. baker's yeast was mixed with 8 ml dd$H_20$, and 0.5 g (~0.5 ml) aliquots of the resulting paste were centrifuged at low rpm. to the bottom of 2ml. Eppendorf tubes. 15 µl of stock 20E (10 mg/ml in ethanol), or of ethanol alone (control), was then mixed into each Eppendorf for a final 20E concentration of 0.3 mg/ml and the yeast smeared into fly vials containing agar medium (11 g agar in 1L dd$H_20$, with 0.044 g Penicillin, 0.133 g Streptomycin, 1.11 g Nipagin and 4 ml Propionic acid). ~15 stalled larvae were added to each fly vial, allowed to feed for 18 hrs, and then dissected and fixed for staining.

**ELISA quantiation of humoral 20E titer.**

Stalled and control larvae were collected in groups of 10, weighed, placed in methanol and stored at -70 °C. Samples were dounce-homogenized, dried by evaporation and resuspended in 500 µl EIA buffer (Cayman Chemicals). The ELISA procedure was carried out with 20E EIA antiserum and 20E acetylcholinesterase (Cayman Chemicals) according to supplier's protocol and using a colorimetric plate reader set between 405 and 414 nm.

## Supplemental Figures

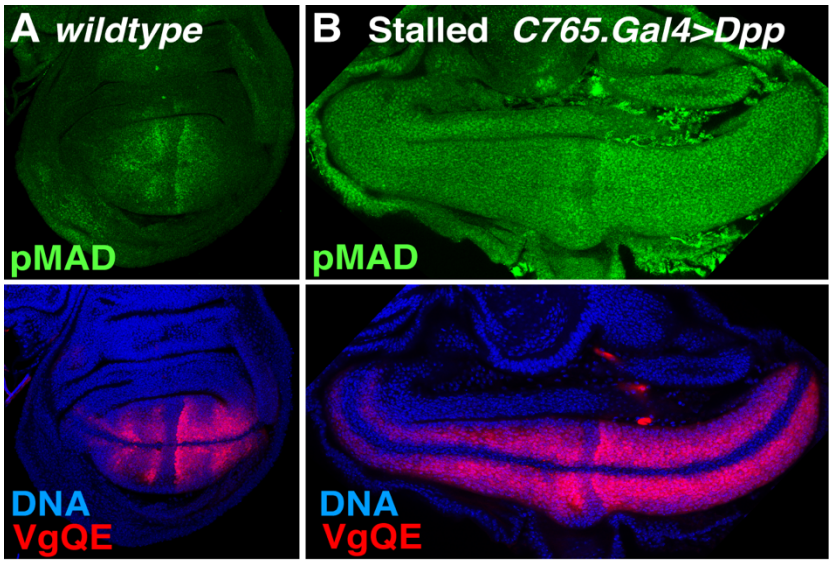


**Figure S1.**

**pMad staining in stalled discs peppered with cells expressing Dpp .**

**A**) Control, *wildtype* disc showing opposing, local gradients of pMAD staining (green) flanking Dpp expressing cells at the AP compartment boundary [*5xQE.Red* expression (red) labels the wing proper; DNA (Hoechst staining) is shown in blue]. Note that the *5xQE.Red* levels increase locally in the vicinity of the prospective wing veins and that pMad accumulation is down-regulated in A cells along the A/P boundary in response to peak Hh signaling. **B)** Stalled larvae (*phm.lexA:VP16 LexOP.fh*$^{RNAi}$) carrying the *hs.flp*, *UAS>CD2, y+>dpp*, and *C765.Gal4* transgenes, heat-shocked for 1 hr. to induce blanket Dpp activity, and dissected 72 hrs later. pMAD staining is elevated throughout the A and P compartments, except for its down-regulation along the A/P boundary, and the wing primordium (*5xQE.DsRed*, red) is dramatically expanded both anteriorly and posteriorly.

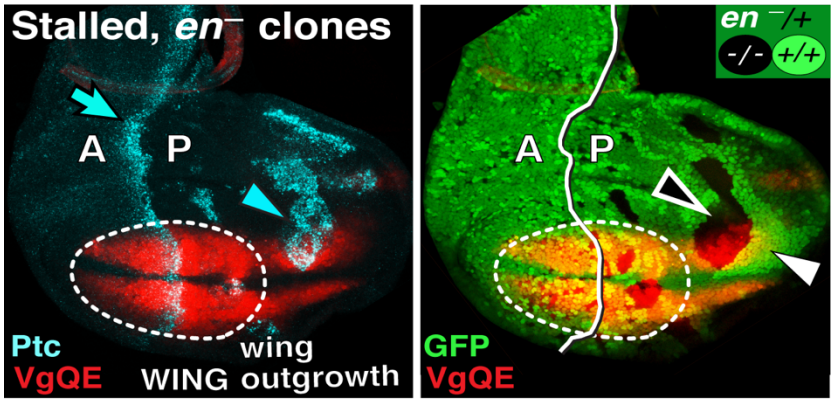


**Figure S2.**

**Clones of *engrailed*⁻ cells in stalled discs sustain outwards growth of the wing from the posterior edge.**

Wing disc from a *phm.lexA:VP16 LexOP.fh*$^{RNAi}$ larva 120 hrs. after heat-shock induction of *en*⁻ clones (by *hsp70.flp* in a *FRT42D en*⁻*/FRT42D Ubi.GFP* transheterozygous background). *en*⁻ clones, marked "black" by the absence of GFP expression (green, right panel) were induced using the same experimental paradigm employed to make *ptc*⁻ clones in **Fig. 3D**, except heat-shock was at 4 instead of 5 days AEL to lessen potential rescuing activity by perduring En protein in the mutant cells. The loss of *en* function transforms P cells into A cells, causing them to autonomously express Dpp as well as the Hh receptor, Ptc (turquoise, arrow head), in response to Hh signaling from neighboring *wildtype* P cells [as normally occurs in A cells that abut P cells across the A/P compartment boundary, arrow; (13)]. As a consequence, the P compartment of the wing continues to expand outwards from the posterior edge, as indicated by the progressive increase in the size of the *en*⁻*/en*⁻ clones and their corresponding 2X *Ubi.GFP* twin spots (marked by brighter intensity GFP staining). By contrast, clones in both compartments within the central portion of the wing, as well as in the remaining portions of the entire A compartment are much smaller, indicating that proliferation arrested within 1-2 days after clone induction at 4 days AEL.

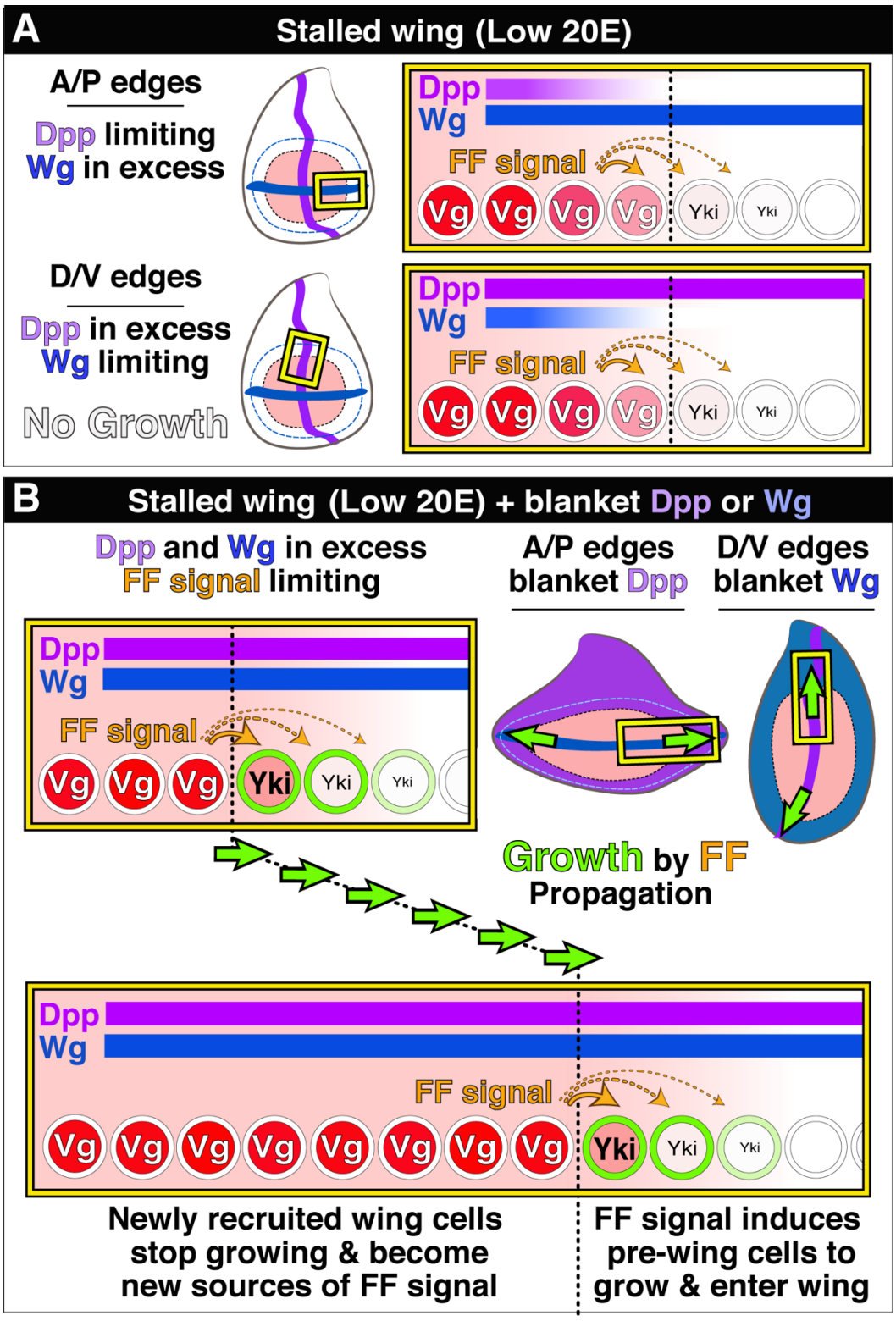


**Figure S3.**

**Ectopic morphogen fuels wing growth by FF propagation.**

**A)** Stalled discs. Top disc: pre-wing cells abutting the anterior and posterior edges of the wing receive high levels of Wg from D/V border cells but are limited for Dpp emanating from A compartment cells along the A/P boundary, preventing their growth and recruitment into the wing (the posterior edge of wing is boxed and shown expanded to the right: high level Wg is depicted by a uniform blue bar and limiting Dpp by the magenta gradient bar). Bottom disc: pre-wing cells at the dorsal and ventral edges of the wing receive high levels of Dpp but fail to grow or enter the wing because they are limited for Wg (dorsal edge is boxed and Dpp and Wg input within the boxed region depicted to the right). **B)** Supplying blanket Dpp or Wg to stalled discs overcomes these local limitations, exposing pre-wing cells at the A/P and D/V edges, respectively, to high levels of both morphogens [depicted as in (**A**)]. Under these conditions, wing growth is limited only by access to the short-range Vg-dependent FF signal, which activates Yki activity in neighboring pre-wing cells and induces them both to grow (green) and to express Vg (red), recruiting them into the wing. Once pre-wing cells enter the wing they cease growing, but become new sources of FF signal, creating a propagating wave front of pre-wing growth and recruitment that expands the wing primordium along the A/P (Dpp) or D/V (Wg) axis, as observed (**Fig. 3**).

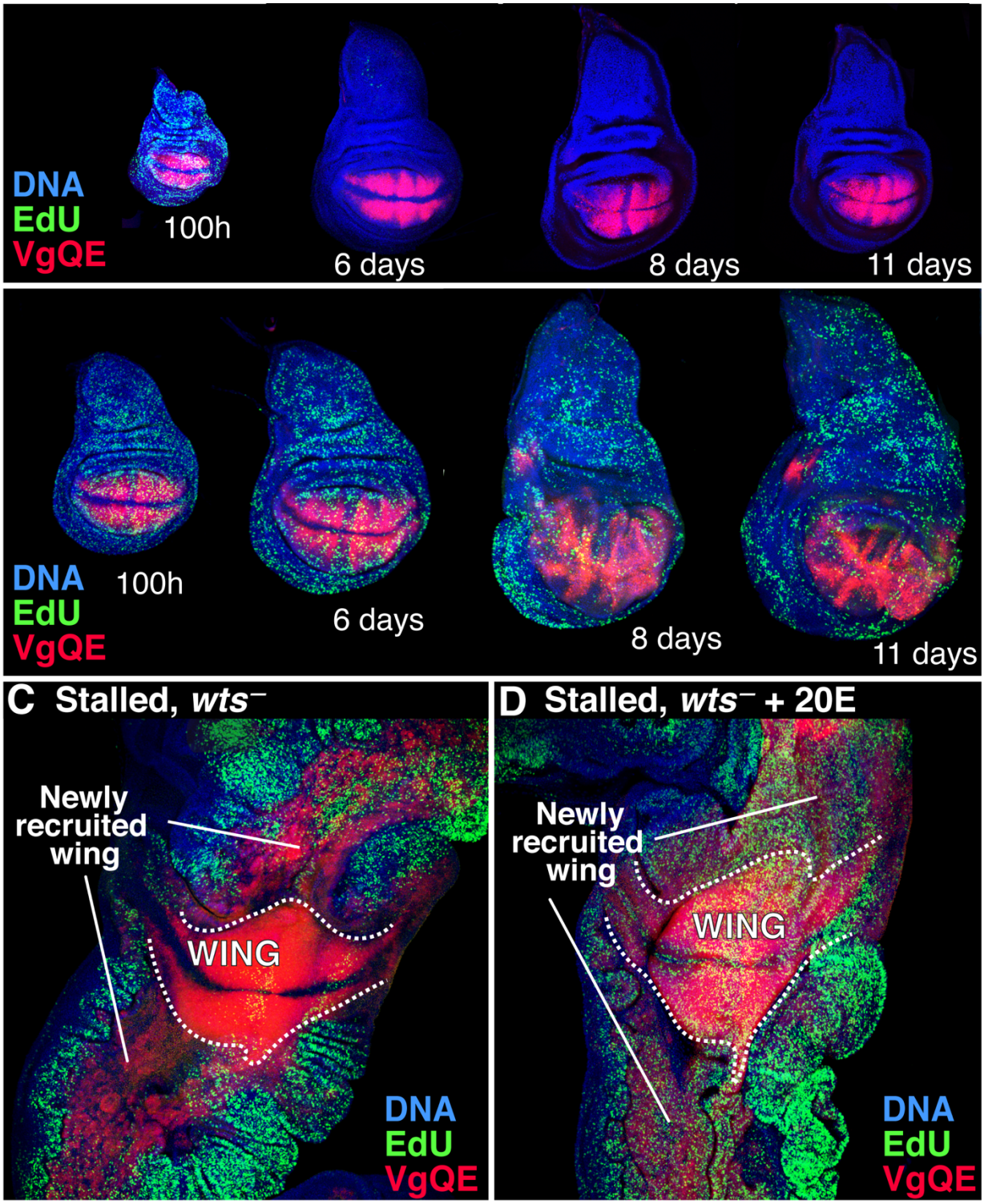


**Figure S4.**

***Usp* RNAi knock-down and 20E feeding sustain growth of the wing proper in both *wildtype* and *wts* mutant stalled discs**

**A, B)** Time course of proliferative growth in *wildtype* wing discs in *phm.lexA:VP16 LexOP.fh*$^{RNAi}$

larvae that either do or do not express $Usp^{RNAi}$ under the disc-specific *C765.Gal4* driver. $Usp^{RNAi}$ knock-down causes wing cells that would otherwise stall (**A**), to continue to proliferate [(**B**); wing cells are marked by *5XQE.DsRed* expression (red), proliferation is monitored by EdU incorporation (green), and DNA is stained by Hoechst (DAPI, blue); as in $EcR^{RNAi}$ expressing larvae (**Fig. 5C**), $Usp^{RNAi}$ expressing larvae continue to feed and gain mass like *phm.lexA:VP16 LexOP.*$fh^{RNAi}$ larvae that do not express $Usp^{RNAi}$, and the discs do not evert, indicating that they do not initiate metamorphosis]. **C,D)** *wts* mutant stalled discs from a control *phm.lexA:VP16 LexOP.*$fh^{RNAi}$ larvae larva (**C**), and a sibling larva fed 20E for 18 hrs. (**D**), stained as in (**A,B**). Proliferation in control $wts^{-}$ stalled discs is largely confined to the population of surrounding the wing proper (**C**), but reinitiates in wing cells of $wts^{-}$ stalled discs following 20E feeding (**D**).
.

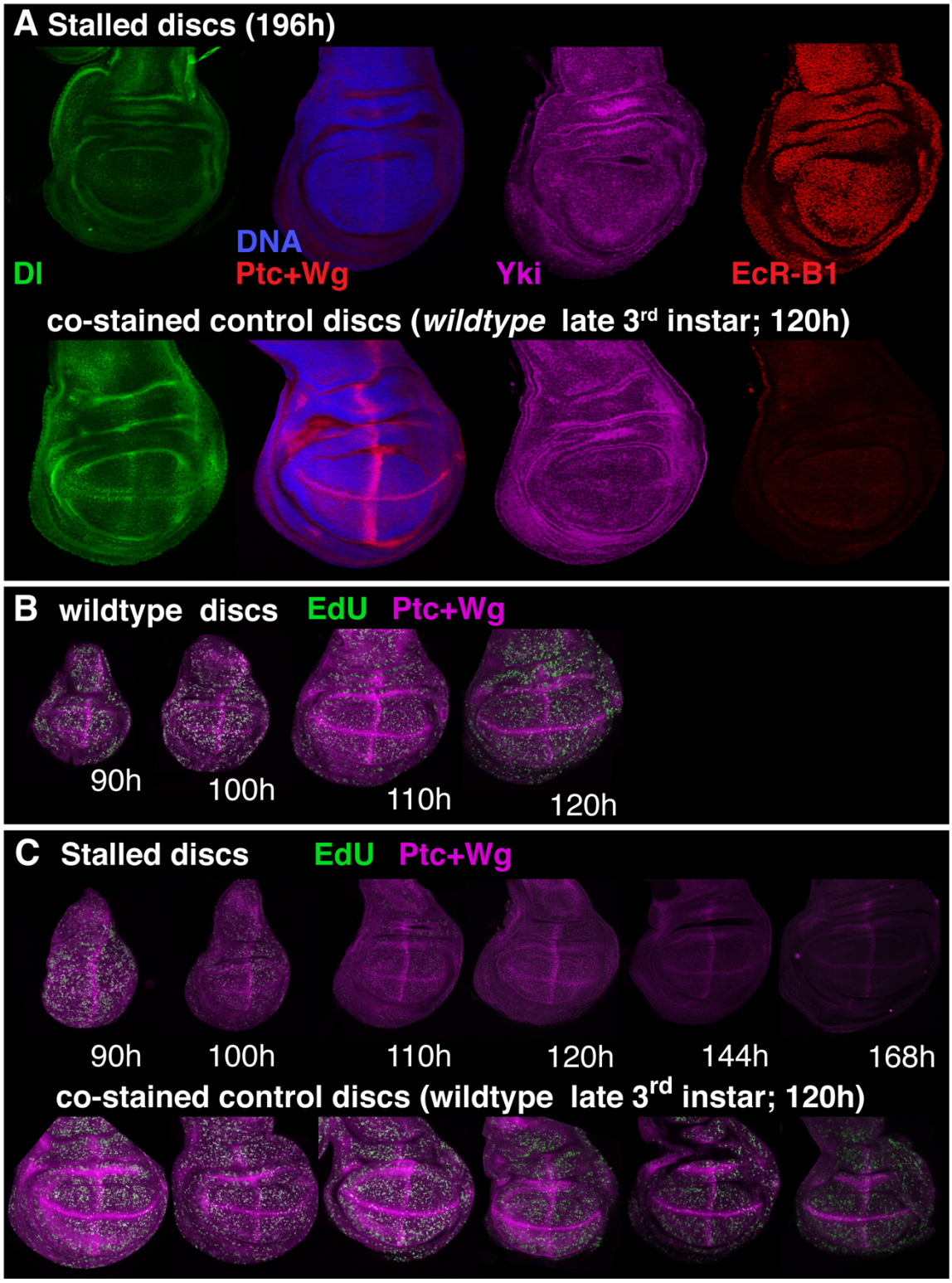
A Stalled discs (196h)
DNA
Dl
Ptc+Wg
Yki
EcR-B1
co-stained control discs (wildtype late 3rd instar; 120h)
B wildtype discs EdU Ptc+Wg
90h
100h
110h
120h
C Stalled discs EdU Ptc+Wg
90h
100h
110h
120h
144h
168h
co-stained control discs (wildtype late 3rd instar; 120h)

**Figure S5.**

**Reduced Hedgehog, Delta and Wg in stalled discs.**

**A)** 8-day old stalled discs (top row) fixed and stained in the same tube and imaged under identical conditions as normally growing, late third instar discs (bottom row; distinguished by *ap.Gal4* driven expression of GFP (as in **Figs 2G,H**, but not shown). The expression of Delta (Dl, green), a Notch ligand required to induce Wg production in D/V border cells as well as that of Wg (red), are greatly diminished in stalled discs; the same is true of Patched (Ptc, red), the receptor for Hedgehog, which is normally up-regulated like Dpp along the A/P border in response to Hh input. All three observations corroborate the results in **Fig. 6**, indicating that Dpp and Wg production are reduced in stalled discs relative to normal third instar discs prior to pupation. By contrast, Yki accumulation (magenta) is unchanged, and the B1 isoform of the EcR (red) is strongly up-regulated, indicating that protein expression is not generally compromised in stalled discs. **B, C)** Time course of EdU incorporation (green) and Ptc+Wg expression (magenta) in wildtype discs (**B**) between 90 and 120 hrs., and in stalled discs between 90 and 168 hrs [top row in (**C**), relative to co-stained, late third instar control discs, bottom row]. While EdU incorporation and Ptc+Wg expression remain relatively constant in wildtype discs between 90 and 120 hrs., both decline concomitantly in stalled discs during the same period, and by 144-168 hrs., EdU incorporation is undetectable and Ptc and Wg expression are severely diminished.

**Table S1**

**Transgene designations of mutant alleles and clone markers in Flybase (https://flybase.org/)**

| | |
|---|---|
| *phm.Gal4* | *P{phm-Gal4.O}3* |
| *C765.Gal4* | *P{GawB}C765* |
| *Act>CD2>Gal4* | *P{Gal4-Act5C(FRT.CD2).P}*) |
| *ap.Gal4* | *P{GawB}ap$^{md544}$* |
| *rn.Gal4* | *P{GawB}ap$^{Gal4}$* |
| *Tubα1.Gal80$^{ts}$* | *P{tubP.Gal80ts}10* |
| *UAS.fh$^{RNAi}$* | *P{UAS.fh.IR}2* |
| *UAS>CD2,y+>dpp* | *P{UAS(FRT.y+CD2)dpp.N}* |
| *UAS>CD2 y+>wg$^{Nrt}$* | *P{UAS(FRT.y+CD2)wg$^{Nrt}$}* |
| *UAS.dcr* | *P{UAS-Dcr-2,D}1* |
| *UAS.EcR$^{RNAi}$* | *P{UAS.EcR-RNAi}104* |
| *UAS.Usp$^{RNAi}$* | *P{TRiP.JF02546}attP2* |
| *UAS.EcR$^{DN}$* | *P{UAS-EcR.B1-ΔC655.W650A}TP1-9* |
| *UAS.GFP* | generic |
| *hsp70.flp* | *P{hsp70.FLP}* |
| *dpp.lacZ* | *P{PZ}dpp10638* |
| *arm-lacZ's* | *P{arm-lacZ.V}36BC, P{arm-lacZ.V}51D* |
| *Ubi.GFP's* | *P{Ubi-GFP(S35T)nls}2R, P{Ubi-GFP.D}83* |
| *VgBE.lacZ* | *P{vg(D/V)-lacZ}* |
| *5XVgQE.DsRed* | *P{5XQE.DsRed}* |
| *FRT's* | *P{neoFRT}40A*, *P{neoFRT}42D*, *P{neoFRT}82B* |

**Table S2**

**Experimental genotypes**

| | |
|---|---|
| Fig. 1B | *VgBE.lacZ/+* |
| Fig. 1C | *5XVgQE.DsRed/+* or *Y*; *VgBE.lacZ/+* |
| Fig. 1D | *5XVgQE.DsRed/+* or *Y* |
| Figs. 2A-E | *UAS.dcr/+* or *Y*; *UAS.fh$^{RNAi}$/+*; *phm.Gal4/+* |
| Figs. 2F-H | *ap.G4 UAS.GFP/+; phm.LexA:VP16/LexOP.fh$^{RNAi}$* |
| Figs. 2G,H | *ap.G4 UAS.GFP/+* (wild type) and *LexOP.fh$^{RNA}$/phm.LexA:VP16* (stalled) discs co-stained |
| Fig. 3B | *hsp70.flp 5XVgQE.DsRed/+* or *Y; UAS>CD2,y+>dpp LexOP.fh$^{RNA}$/phm.LexA:VP16 6 C765.Gal4* |
| Fig. 3C | *hsp70.flp 5XVgQE.DsRed/+* or *Y; FRT40A/arm.lacZ FRT40A; UAS>CD2,y+>dpp LexOP.fh$^{RNA}$/phm.LexA:VP16 C765.Gal4* |
| Fig. 3E | *hsp70.flp 5XVgQE.DsRed/+* or *Y; FRT42D ptc$^{16}$/FRT42D Tubα1.DsRed; LexOP.fh$^{RNA}$/phm.LexA:VP16* |
| Fig. 3G | *hsp70.flp 5XVgQE.DsRed/+* or *Y; LexOP.fh$^{RNA}$ UAS>CD2,y+>wg$^{Nrt}$/phm.LexA:VP16 C765.Gal4* |
| Fig. 3H | *hsp70.flp 5XVgQE.DsRed/+* or *Y; FRT40A/arm.lacZ FRT40A; UAS>CD2,y+>wg$^{Nrt}$ LexOP.fh$^{RNA}$/phm.LexA:VP16 C765.Gal4* |
| Fig. 4A | *5XVgQE.DsRed/+* or *Y; LexOP.fh$^{RNA}$/phm. LexA:VP16* |
| Figs. 4B,D,F | *5XVgQE.DsRed/+* or *Y; LexOP.fh$^{RNA}$ wts$^{P2}$/phm.LexA:VP16 wts$^{P2}$* |
| Fig. 4E | *5XVgQE.DsRed/+* or *Y; wg$^{spd\text{-}flg}$/wg$^{spd\text{-}flg}$; LexOP.fh$^{RNA}$ wts$^{P2}$/phm.LexA:VP16 wts$^{P2}$* |
| Fig. 4G,H | *hsp70.flp 5XVgQE.DsRed/+* or *Y; dpp$^{d8}$ nubbin.GAL4/dpp$^{d10}$ UAS>CD2,y+>dpp$^{GFP}$; LexOP.fh$^{RNA}$ wts$^{P2}$/phm.LexA:VP16 wts$^{P2}$* (heat-shocked in H, but not in G) |
| Figs. 5A,E-G | *LexOP.fh$^{RNA}$/phm. LexA:VP16* |
| Fig. 5B | *5XVgQE.DsRed/+* or *Y; LexOP.fh$^{RNA}$/+ ; phm.LexA:VP16 C765.Gal4/ +* |
| Fig. 5C | *5XVgQE.DsRed/+* or *Y; LexOP.fh$^{RNA}$ UAS.EcR$^{RNAi}$/phm.LexA:VP16 C765.Gal4* |

| | |
|---|---|
| Fig. 5D | *UAS.EcR$^{RNAi}$/rn.Gal4* |
| Fig. 5I-K | *hsp70.flp; Tubα1.Gal80$^{ts}$/+; Act>CD2>Gal4 UAS.GFP/UAS.EcR$^{DN}$* |
| Fig. S1A | *5XVgQE.DsRed/+* or *Y* |
| Fig. S1B | *hsp70.flp 5XVgQE.DsRed/+* or *Y; UAS>CD2,y+>dpp LexOP.fh$^{RNA}$/phm.LexA:VP16 6 C765.Gal4* |
| Fig. S2 | *hsp70.flp 5XVgQE.DsRed/+* or *Y; FRT42D en$^{E}$/ FRT42D Ubi.GFP; LexOP.fh$^{RNA}$/phm.LexA:VP16* |
| Fig. S4A | *5XVgQE.DsRed/+* or *Y; LexOP.fh$^{RNA}$/phm.LexA:VP16* |
| Fig. S4B | *5XVgQE.DsRed/+* or *Y; LexOP.fh$^{RNA}$ UAS.Usp$^{RNAi}$/phm.LexA:VP16 C765.Gal4* |
| Fig. S4C,D | *5XVgQE.DsRed/+* or *Y; LexOP.fh$^{RNA}$ wts$^{P2}$/phm.LexA:VP16 wts$^{P2}$* |
| Fig. S5 | *LexOP.fh$^{RNA}$/phm.LexA:VP16* (stalled) and *ap.G4 UAS.GFP/+* (wild type) discs co-stained |

**SI References**


1. M. Zecca, G. Struhl, Recruitment of cells into the Drosophila wing primordium by a feed-forward circuit of vestigial autoregulation. *Development* **134**, 3001–3010 (2007).

2. H. Ono, *et al.*, Spook and Spookier code for stage-specific components of the ecdysone biosynthetic pathway in Diptera. *Dev Biol* **298**, 555–570 (2006).

3. C. Estella, D. J. McKay, R. S. Mann, Molecular integration of wingless, decapentaplegic, and autoregulatory inputs into Distalless during Drosophila leg development. *Dev Cell* **14**, 86–96 (2008).

4. S.-L. Lai, T. Lee, "Genetic mosaic with dual binary transcriptional systems in Drosophila" (Nature Publishing Group, 2006).

5. M. Markstein, C. Pitsouli, C. Villalta, S. E. Celniker, N. Perrimon, Exploiting position effects and the gypsy retrovirus insulator to engineer precisely expressed transgenes. *Nat Genet* **40**, 476–483 (2008).

6. J. Bischof, R. K. Maeda, M. Hediger, F. Karch, K. Basler, An optimized transgenesis system for Drosophila using germ-line-specific phiC31 integrases. *Proc Natl Acad Sci USA* **104**, 3312–3317 (2007).

7. P. R. Anderson, RNAi-mediated suppression of the mitochondrial iron chaperone, frataxin, in Drosophila. *Hum Mol Genet* **14**, 3397–3405 (2005).

8. R. T. Coleman, G. Struhl, Causal role for inheritance of H3K27me3 in maintaining the OFF state of a DrosophilaHOX gene. *Science* **356**, eaai8236 (2017).

9. M. Zecca, K. Basler, G. Struhl, Direct and long-range action of a wingless morphogen gradient. **87**, 833–844 (1996).

10. M. Zecca, G. Struhl, Control of Drosophila wing growth by the vestigial quadrant enhancer. *Development* **134**, 3011–3020 (2007).

11. L. A. Baena-López, X. Franch-Marro, J.-P. Vincent, Wingless promotes proliferative growth in a gradient-independent manner. *Science signaling* **2**, ra60 (2009).

12. Z. McBrayer, *et al.*, Prothoracicotropic hormone regulates developmental timing and body size in Drosophila. *Dev Cell* **13**, 857–871 (2007).

13. T. Tabata, C. Schwartz, E. Gustavson, Z. Ali, T. B. Kornberg, Creating a Drosophila wing de novo, the role of engrailed, and the compartment border hypothesis. *Development* **121**, 3359–3369 (1995).